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Year: 2009

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## Phenotyping of N-acetyltransferase type 2 and xanthine oxidase with caffeine: when should urine samples be collected?

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**Abstract:** OBJECTIVES: Individual activities of N-acetyltransferase 2 (NAT2) and of xanthine oxidase (XO) can be assessed using ratios of urinary caffeine metabolites. We investigated how ratios changed over time and which urine collection interval would be the best for NAT2 and XO activity assessments. METHODS: On two occasions separated by 14 days, 16 healthy male Caucasians collected urine before and 0-2, 2-4, 4-6, 6-8, 8-12, 12-16 and 16-24 h after a dose of 150 mg caffeine given in the framework of a phenotyping cocktail study. The metabolites 5-acetylamino-6-formylamino-3-methyluracil (AFMU), 5-acetylamino-6-amino-3-methyluracil (AAMU), 1-methylxanthine (1X), and 1-methylurate (1U) were quantified with LC-MS/MS. The molar ratio  $(AFMU + AAMU)/(1X + 1U + AFMU + AAMU)$  was used as a NAT2 metric, while the ratio  $1U/(1X + 1U)$  served as XO metric. RESULTS: The NAT2 ratios were stable in the intervals 4-24 h after caffeine dosing. Mean intra-individual coefficients of variation were 11-23% starting 4 h post-dose, while inter-individual variability reached 37-75%. The XO ratios increased gradually by 14% from the 2-4 to the 16-24 h interval. The mean intra- and inter-individual coefficients of variation of XO activity were 3-18 and 7-10% respectively. No significant differences between study occasions were observed. CONCLUSIONS: Any sampling interval at least 4 h after caffeine dosing is suitable for NAT2 and XO activity assessments. XO activities can only be compared between volunteers and studies if the same urine collection schedule has been respected. The low intraindividual variability allows for sample sizes of 16 and 6 participants in crossover interaction studies of NAT2 and XO activity respectively.

DOI: <https://doi.org/10.1007/s00228-008-0597-0>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-8830>

Journal Article

Published Version

Originally published at:

Jetter, A; Kinzig, M; Rodamer, M; Tomalik-Scharte, D; Sörgel, F; Fuhr, U (2009). Phenotyping of N-acetyltransferase type 2 and xanthine oxidase with caffeine: when should urine samples be collected? *European Journal of Clinical Pharmacology*, 65(4):411-417.

DOI: <https://doi.org/10.1007/s00228-008-0597-0>

# Phenotyping of *N*-acetyltransferase type 2 and xanthine oxidase with caffeine: when should urine samples be collected?

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Received: 12 September 2008 / Accepted: 24 November 2008 / Published online: 13 December 2008  
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## Abstract

**Objectives** Individual activities of *N*-acetyltransferase 2 (NAT2) and of xanthine oxidase (XO) can be assessed using ratios of urinary caffeine metabolites. We investigated how ratios changed over time and which urine collection interval would be the best for NAT2 and XO activity assessments.

**Methods** On two occasions separated by 14 days, 16 healthy male Caucasians collected urine before and 0–2, 2–4, 4–6, 6–8, 8–12, 12–16 and 16–24 h after a dose of 150 mg caffeine given in the framework of a phenotyping cocktail study. The metabolites 5-acetylamino-6-formylamino-3-methyluracil (AFMU), 5-acetylamino-6-amino-3-methyluracil (AAMU), 1-methylxanthine (1X), and 1-methylurate (1U) were quantified with LC-MS/MS. The molar ratio  $(AFMU + AAMU)/(1X + 1U + AFMU + AAMU)$  was used as a NAT2 metric, while the ratio  $1U/(1X + 1U)$  served as XO metric.

**Results** The NAT2 ratios were stable in the intervals 4–24 h after caffeine dosing. Mean intra-individual coefficients of variation were 11–23% starting 4 h post-dose, while inter-individual variability reached 37–75%. The XO ratios increased gradually by 14% from the 2–4 to the 16–24 h interval. The mean intra- and inter-individual coefficients of variation of XO activity were 3–18 and 7–10% respectively. No significant differences between study occasions were observed.

**Conclusions** Any sampling interval at least 4 h after caffeine dosing is suitable for NAT2 and XO activity assessments. XO activities can only be compared between volunteers and studies if the same urine collection schedule has been respected. The low intraindividual variability allows for sample sizes of 16 and 6 participants in crossover interaction studies of NAT2 and XO activity respectively.

**Keywords** *N*-acetyltransferase 2 · Xanthine oxidase · Phenotyping · Caffeine · Intra-individual variability

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## Introduction

The genetically polymorphic phase-II enzyme *N*-acetyltransferase type 2 (NAT2) is important for the metabolism of drugs such as isoniazid and several sulfonamides and is involved in the bioactivation of several procarcinogens, e.g. from tobacco smoke [1]. More than 50 allelic variants have been described, and approximately 50% of Caucasians are classified as slow acetylators.

The enzyme xanthine oxidase (XO) plays a crucial role in many metabolic processes: it produces reactive oxygen species, catalyses uric acid production and plays a role in the elimination of thiopurine drugs [2–4]. About 20 genetic

variants are known [5], but in studies, 4% or less of volunteers examined showed decreased enzyme activity [6]. However, xanthine oxidase activity is also influenced by endogenous compounds, other drugs (e.g. inhibition by the allopurinol metabolite oxypurinol or induction by cytokines or ethacrynic acid) and environmental factors [2, 3].

Since the 1980s, the quantification of urinary caffeine metabolites has been used frequently to assess the actual activities of NAT2 [7, 8] and XO [9]. A variety of different metabolite ratios, sampling schemes and schedules have been developed and are used in different study settings. Not every approach has been fully validated, so the results may not always be reliable and comparable. Generally, NAT2 activity assessment has attracted more attention than XO activity estimation. It is therefore important to know whether differences in the phenotyping procedures have a relevant effect on the results and reduce their comparability. In this context, we showed earlier that caffeine from uncontrolled dietary sources may be used for NAT2 phenotyping if a correct abstinence period is respected [10]. The present work was carried out to investigate whether the timing of urine collections, which differs considerably across publications, has a relevant influence on NAT2 and XO phenotype assessments, and whether intra- and inter-individual variability changes as a function of (urine collection) time. Since intra-individual variability is a crucial factor for the correct estimation of sample sizes in crossover interaction studies, we determined the numbers of participants necessary for the reliable estimation of changes in NAT2 and XO activities in such a study setting.

## Methods

### Study conduct and subjects

This investigation is based on urine samples collected during a two-period, randomised crossover interaction study in 16 healthy, young, non-smoking male Caucasians. The study was carried out to assess the effects of propiverine on the activities of hepatic CYP3A (using intravenous midazolam), intestinal CYP3A (oral midazolam), CYP2C9 (tolbutamide), CYP2C19 (mephenytoin), and CYP1A2 (150 mg caffeine; Percodrinol tablets, Passauer, Berlin, Germany) and which has been published earlier [11].

All volunteers gave their written informed consent before the screening examination. The study was endorsed by the Ethics Committee of the University of Cologne, Germany, and conducted according to the principles of good clinical practice and the laws of Germany.

The cocktail was given under controlled in-house conditions on day 7 of regular administration of 15 mg propiverine or placebo twice daily. A washout period of

14 days was respected between the two cocktail administrations. Any methylxanthine-containing food or beverage was forbidden from 72 h before the first drug intake until 48 h after the last dosing. In addition to blood collections for the main purposes of the study, urine was collected before and at 0–2, 2–4, 4–6, 6–8, 8–12, 12–16 and 16–24 h after caffeine intake. The urine containers were kept at +4°C during the collection periods, and the participants were instructed to void all urine during the collection periods in the containers. Weight and pH of the urine collection were recorded, and acidified samples (1 mL 6 M HCl) were stored at –20°C until analysis.

### Quantification of caffeine metabolites in urine

The caffeine metabolites 5-acetylamino-6-formylamino-3-methyluracil (AFMU), 5-acetylamino-6-amino-3-methyluracil (AAMU), 1-methylxanthine (1X) and 1-methylurate (1U) were quantified with a fully validated LC-MS/MS method as described earlier without modification [10]. The lower limit of quantification was 100 ng/mL for all analytes except for AAMU, where it was 200 ng/mL.

### Statistical analysis

For each sampling interval, the molar urinary ratios (AFMU+AAMU)/(AFMU+AAMU+1X+1U) were calculated as parameters of NAT2 activity [12]. As parameters of XO activity, the molar urinary ratios (1U)/(1X+1U) were calculated [13]. To obtain estimates of intra-individual and inter-individual variability, linear mixed models were established using maximum likelihood estimation based on ln-transformed ratios of each collection period that took “sequence”, “period”, and “comedication” as fixed effects, and “volunteer (sequence)” as random effect into account. The coefficient of variation of the multiplicative model from the residual variance was considered as the intra-individual variability, while the multiplicative coefficient of variation obtained from the covariance of the random effect “volunteer (sequence)” served as an estimate of the inter-individual variability.

For the comparisons between sampling intervals, linear mixed-effect models were fitted to assess the influence of collection time on the variability between collection periods. In these models, timing of collection intervals was handled as a continuous variable and included as an additional fixed effect. Statistical significance was accepted for  $P \leq 0.05$ . The intra-individual CVs were used to estimate the sample size needed for a crossover interaction study with the NAT2 and XO metrics under the assumption that the ratio  $\mu_{\text{test}}/\mu_{\text{reference}}$  is in the 0.95–1.05 interval with 90% confidence intervals between 0.80 and 1.25, a power of 0.80 and  $P=0.05$  [14]. SPSS 15.0.0 for windows (SPSS,

Chicago, IL, USA), Excel 2003 (Microsoft, Redmond, WA, USA), and SigmaPlot 10.0 (Systat Software, San Jose, CA, USA) were used for statistical and graphical analysis.

## Results

### N-acetyltransferase 2

The urinary metabolite ratios which were used to assess NAT2 activity were bimodally distributed starting with the 4–6 h collection period through the 16–24 h collection period (Fig. 1). In the ANOVA-based mixed effect modelling using ln-transformed metabolic ratios of these collection intervals, neither the timing of the urine collection ( $P=0.60$ ) nor the period ( $P=0.28$ ) or sequence ( $P=0.38$ ) was a significant factor of influence, while

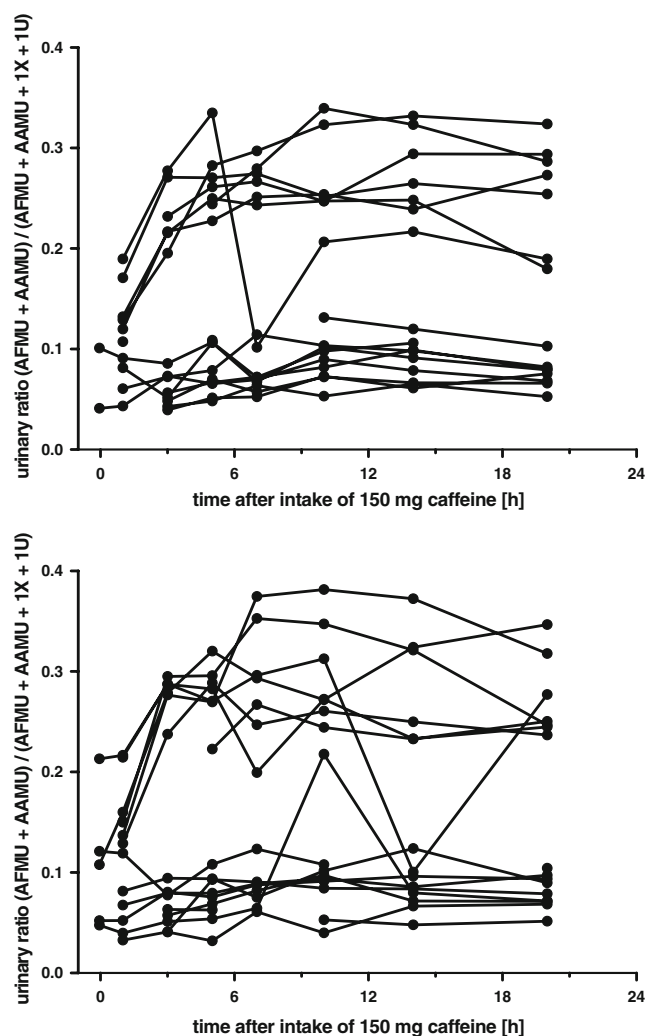
comedication weakly influenced the overall model ( $P=0.04$ ). The overall intra-individual variability was 19.9%, while inter-individual variability reached 67.0%. The modelling of the individual collection periods from 4 to 24 h post-dose yielded intra-individual coefficients of variation of the metabolic ratios between 10.5% (collection interval 16–24 h post-dose) and 23.3% (collection interval 8–12 h post-dose), while inter-individual CVs ranged between 36.5 and 74.8% (Table 1). No statistically significant influence of comedication, period, or sequence on the NAT2 ratios was observed in any model of the individual collection periods.

Rapid and slow acetylators were well distinguished (Figs. 1 and 2). Their respective overall mean metabolic ratios in urine collected between 4 and 24 h after 150 mg caffeine were 0.269 (CV, 18.7%) for rapid and 0.079 (CV, 31.9%) for slow acetylators. The acetylation phenotype did not change between the periods (Fig. 2). It has to be noted, however, that in three cases, ratios in isolated sampling intervals were outside the range of their individual values and their group means. Urine samples collected before the caffeine test dose from two and five volunteers during periods 1 and 2 respectively contained concentrations of AFMU and/or AAMU which were above the quantification limit. Since the intra-individual CV was 17.5% during the 4–6 h collection interval, a sample size of  $n=16$  volunteers appeared appropriate for crossover interaction studies using this parameter ( $\alpha=0.05$ , power 80%, true  $\mu_{\text{test}}/\mu_{\text{reference}}$  in the 0.95–1.05 range, “no effect” boundaries 0.8–1.25).

### Xanthine oxidase

The ratios representing XO activity were unimodally distributed. In the overall linear mixed effect model, time was a significant factor of influence ( $P<0.00001$ ) which led to an increase in ratios by 14.5% from the 2–4 h collection interval to the 16–24 h collection interval (Fig. 3). While sequence ( $P=0.90$ ) and comedication ( $P=0.36$ ) did not influence the ratios in the model, XO ratios during period 1 were 3.1% higher than during period 2 ( $P=0.024$ ). In this model, the inter-individual variability reached 8.2%, while the global intra-individual coefficient of variation was 9.2%. The arithmetic means and inter- and intra-individual coefficients of variation for the individual collection periods are shown in Table 1. The lowest intra-individual coefficient of variation was 2.6%, observed in the collection interval 2–4 h post-dose, while the highest was 18.2%, observed in the collection interval 6–8 h after caffeine intake. The mean interindividual coefficients of variation ranged between 6.5 and 10.3% (Table 1).

In the individual models for each collection period, no statistically significant influences of the factors study period



**Fig. 1** Time courses of individual NAT2 metabolic ratios in 16 healthy non-smokers in the first (*above*) and second (*below*) period of a crossover study. Time values represent the middle of collection intervals

**Table 1** Arithmetic means, inter-individual ( $CV_{inter}$ ) and intra-individual coefficients of variation ( $CV_{intra}$ ) for the urinary molar ratios for NAT2 and XO activity

Sampling interval	NAT2 (all 16 participants)			NAT2 rapid acetylators ( $n=7$ )			NAT2 slow acetylators ( $n=9$ )			Xanthine oxidase ( $n=16$ )		
	Mean	$CV_{inter}$	$CV_{intra}$	Mean	$CV_{inter}$	$CV_{intra}$	Mean	$CV_{inter}$	$CV_{intra}$	Mean	$CV_{inter}$	$CV_{intra}$
Period 1: predose	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	0.820	3.6%	9.4%
Period 2: predose	n.c.			n.c.			n.c.			0.812		
Period 1: 0–2 h	0.112	49.1%	27.6%	0.148	9.1%	14.6%	0.077	29.1%	27.4%	0.520	7.9%	5.5%
Period 2: 0–2 h	0.119			0.165			0.065			0.534		
Period 1: 2–4 h	0.134	82.2%	15.0%	0.235	5.0%	6.8%	0.059	20.5%	14.3%	0.541	6.5%	2.6%
Period 2: 2–4 h	0.150			0.277			0.065			0.549		
Period 1: 4–6 h	0.161	74.8%	17.5%	0.267	7.4%	10.2%	0.078	24.1%	19.5%	0.556	6.9%	3.6%
Period 2: 4–6 h	0.163			0.279			0.074			0.571		
Period 1: 6–8 h	0.152 <sup>a</sup>	68.3%	20.0%	0.245 <sup>a</sup>	10.6%	24.7%	0.071	18.3%	11.8%	0.600 <sup>a</sup>	10.3%	18.2%
Period 2: 6–8 h	0.180			0.290			0.084			0.556 <sup>a</sup>		
Period 1: 8–12 h	0.167	36.5%	23.3%	0.267	2.2%	15.3%	0.089	14.1%	26.7%	0.604	8.7%	6.4%
Period 2: 8–12 h	0.186 <sup>a</sup>			0.299			0.098 <sup>a</sup>			0.580		
Period 1: 12–16 h	0.169	36.6%	16.8%	0.274	22.5%	20.2%	0.087	19.6%	7.9%	0.611	7.4%	8.5%
Period 2: 12–16 h	0.166 <sup>a</sup>			0.262 <sup>a</sup>			0.082			0.583		
Period 1: 16–24 h	0.160	66.6%	10.5%	0.257	9.5%	13.2%	0.076	15.5%	5.5%	0.642	7.8%	4.6%
Period 2: 16–24 h	0.165			0.274			0.081			0.624		

n.c. Not calculated since analytes were below the quantification limit in more than 50% of participants

<sup>a</sup> Including one outlier; see text for details

(Fig. 4), sequence or comedication were observed. Before dosing, the concentrations of 1U were above the quantification limit in the urine of all volunteers, and concentrations of 1X were quantifiable in 13 and 14 urine samples before the first and the second caffeine intake respectively. Taking the same sampling interval and the same conditions for sample size estimation as used for NAT2 into account, the intra-individual variability of XO activity in the 4–6 h collection interval of 3.6% would lead to a sample size of  $n=6$  for crossover interaction studies.

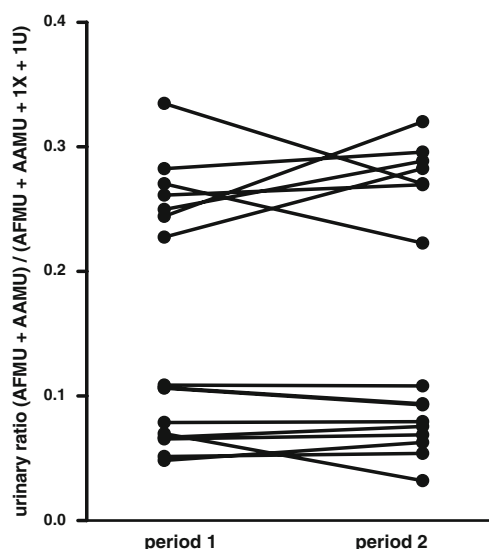
## Discussion

Although many investigations use urinary metabolite ratios for the assessment of NAT2 and XO activities, to our knowledge, the question of the appropriate urine sampling interval, which is of importance for the validity and comparability of results, has not yet been scrutinised. In the present investigation, a short urine sampling interval of 2-h duration was sufficient if more than 4 (NAT2) or 2 h (XO) had elapsed since the caffeine test dose. While for NAT2 phenotyping, the exact choice of collection time after that had no relevant influence on the adequacy of the results, XO activity parameters increased gradually with time. These results may facilitate future research in this area and may help to establish the formal correctness of earlier results.

For NAT2 phenotyping, three ratios based on urinary caffeine metabolites have been proposed [15]. The ratio used in the present investigation is an extension of the ratio

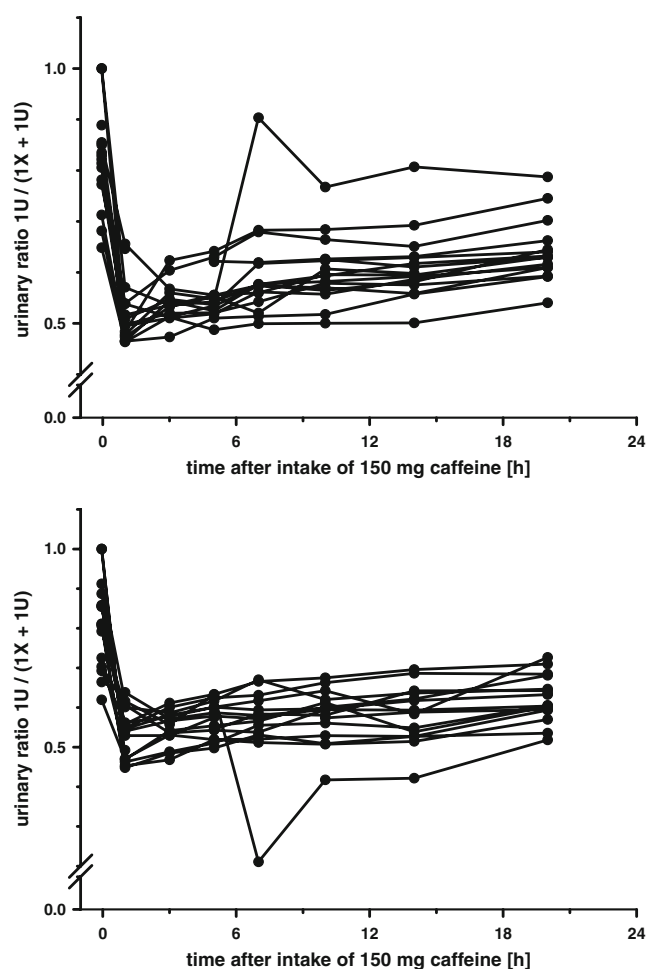
proposed by Tang and co-workers [12] which has been used most frequently and is fairly well validated [10 and references therein]. Since both AFMU and AAMU are included in the formula we used, questions and problems surrounding the deformylation of AFMU [10, 16] were avoided.

For the assessment of XO activity, the ratios 1U/1X and (1U)/(1X+1U) have been used and validated in combination with NAT2 and CYP1A2 assessments [17–21] and in



**Fig. 2** Individual NAT2 activity quantified in urine collected 4–6 h after dosing





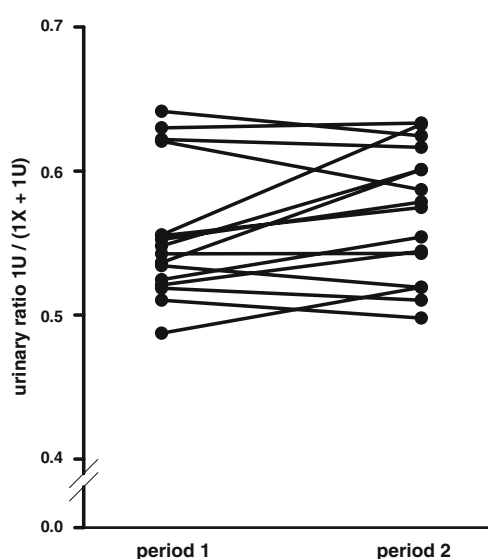
**Fig. 3** Time courses of individual XO metabolic ratios in 16 healthy non-smokers in the first (*above*) and second (*below*) period of a crossover study. Time values represent the middle of collection intervals

the framework of cocktail phenotyping studies [22–24]. No direct comparison between the performance of the two ratios has been published, and for both, some validation issues have not yet been addressed. However, the latter ratio is more easily interpreted (the more 1U is formed and the more active XO is, the closer the values are to 1), and it can still be calculated if all 1X has been converted to 1U. Since 1X appears to be also a substrate for renal organic anion transporters, inhibition of these transporters, e.g. by probenecid, has an influence on the ratio [21, 25, 26].

Traditionally, most studies investigating NAT2 and XO activities use 6-, 8-, or 12-h urine sampling intervals or spot urine samples between 4 and 8 h after intake of the caffeine test dose. While the latter may have advantages in large-scale studies where urine collections over longer periods may be impractical, our data suggest that, if urine collection is carried out, it is sufficient to collect for 2 h at least 4 h after the dosing. For NAT2 phenotyping, the collection interval 4–6 h after caffeine intake may be the best

compromise between accuracy of results (Figs. 1 and 2, Table 1) and practicability for participants and investigators (duration of caffeine abstinence and urine collection). Longer collection periods may bear the risk that caffeine abstinence is not observed. Interestingly, early publications on NAT2 phenotyping denied the need for caffeine abstinence [8, 27].

In the present investigation, we observed intra-individual variabilities in NAT2 ratios between 10.5 and 23.3% from 4 h after dosing, and in XO ratios between 2.6 and 18.2% from 2 h after dosing. Only a few published studies quantify the intra-individual variability of NAT2 or XO. The intra-individual variability is reported to be in a range of 6 to 49% for NAT2 [8, 27] and about 5% for XO [18]. We could substantiate that the intra-individual variability for both NAT2 and XO activity is low, at least under the controlled conditions of a randomised clinical study. This allows, e.g., the conduct of crossover interaction studies with low numbers of participants. However, in three urine collections 6 or more h after caffeine dosing, NAT2 ratios were outside the range of the other individual values and in the range of the opposite acetylator phenotype. For XO, data in two volunteers, one in each period and direction, deviated markedly from the mean starting with the 6–8 h collection interval. These deviations had an impact on the estimations of the intra-individual variabilities in the respective collection periods. Since the first food intake took place 6 h after caffeine dosing, it may be speculated whether food intake had an influence, however, food was standardised, and it remains elusive why outlying values were only observed in one volunteer in each period. The influences of comedication and period which were ob-



**Fig. 4** Individual XO activities quantified in urine collected 4–6 h after dosing

served in the overall models for NAT2 and XO phenotyping respectively were not observed in single collection period models. Hence, these factors did not influence the estimations of the variabilities for the individual collections, and may have been observed by chance.

In predose urine samples of most volunteers, some caffeine metabolites were quantified in low concentrations. It may be speculated that the low quantification limits of our analytical method enabled the quantification of these metabolites which may have resulted from low-dose contamination of food and beverages with methylxanthines. Noncompliance seemed unlikely, since volunteers were under observation the night before the phenotyping day, and they were regularly questioned about adherence to dietary restrictions.

Since for XO activity, both intra- and inter-individual variability was lower than for NAT2 activity, and since samples from 2–24 h after caffeine intake may be used for XO activity assessment, the best choice for XO phenotyping would be to use the same 4–6 h collection interval as used for NAT2 phenotyping (Fig. 4).

In conclusion, the present investigation scrutinised the influence of timing of urine collection intervals on NAT2 and XO activity parameters. Although the use of urine collection 4–6 h after caffeine dosing appears to be the optimum, most other later collection schedules yielded valid results, if at least 4 h (2 h for XO phenotyping) had elapsed. It has to be noted that the timing of the collection interval influences the results of XO phenotyping. Hence, XO activities quantified at different time intervals are not comparable. In consideration of the low intra-individual coefficients of variation, crossover studies which investigate the interaction of a drug with NAT2 or XO using the same ratios as used here may be carried out with as few as 16 and 6 participants respectively.

**Acknowledgements** Parts of this investigation were presented at the 2004 Annual Meeting of the American Society for Clinical Pharmacology and Therapeutics, ASCPT, in Miami, FL, USA. None of the authors has a financial relationship which could potentially have influenced the results.

## References

- Butcher NJ, Boukouvala S, Sim E et al (2002) Pharmacogenetics of the arylamine N-acetyltransferases. *Pharmacogenomics* 2: 30–42
- Pacher P, Nivorozhkin A, Szabo C (2006) Therapeutic effects of xanthine oxidase inhibitors: renaissance half a century after the discovery of allopurinol. *Pharmacol Rev* 58:87–114
- Pritsos CA (2000) Cellular distribution, metabolism and regulation of the xanthine oxidoreductase enzyme system. *Chem Biol Interact* 129:195–208
- Kitamura S, Sugihara K, Ohta S (2006) Drug-metabolizing ability of molybdenum hydroxylases. *Drug Metab Pharmacokinet* 21: 83–98
- Kudo M, Moteki T, Sasaki T et al (2008) Functional characterization of human xanthine oxidase allelic variants. *Pharmacogenet Genomics* 18:243–251
- Aklilu E, Carrillo JA, Makonnen E et al (2003) Xanthine oxidase activity is influenced by environmental factors in Ethiopians. *Eur J Clin Pharmacol* 59:533–536
- Grant DM, Tang BK, Kalow W (1983) Polymorphic N-acetylation of a caffeine metabolite. *Clin Pharmacol Ther* 33:355–359
- Hardy BG, Lemieux C, Walker SE et al (1988) Interindividual and intraindividual variability in acetylation: characterization with caffeine. *Clin Pharmacol Ther* 44:152–157
- Grant DM, Tang BK, Kalow W (1983) Variability in caffeine metabolism. *Clin Pharmacol Ther* 33:591–602
- Jetter A, Kinzig-Schippers M, Illauer M et al (2004) Phenotyping of N-acetyltransferase type 2 by caffeine from uncontrolled dietary exposure. *Eur J Clin Pharmacol* 60:17–21
- Tomalik-Scharte D, Jetter A, Kinzig-Schippers M et al (2005) Effect of propiverine on cytochrome P450 enzymes: a cocktail interaction study in healthy volunteers. *Drug Metab Dispos* 33: 1859–1866
- Tang BK, Kadar D, Qian L et al (1991) Caffeine as a metabolic probe: validation of its use for acetylator phenotyping. *Clin Pharmacol Ther* 49:648–657
- Kalow W, Tang BK (1991) Caffeine as a metabolic probe: exploration of the enzyme-inducing effect of cigarette smoking. *Clin Pharmacol Ther* 49:44–48
- Diletti E, Hauschke D, Steinijans VW (1991) Sample size determination for bioequivalence assessment by means of confidence intervals. *Int J Clin Pharmacol Ther Toxicol* 29:1–8
- Rostami-Hodjegan A, Nurminen S, Jackson PR et al (1996) Caffeine urinary metabolite ratios as markers of enzyme activity: a theoretical assessment. *Pharmacogenetics* 6:121–149
- Ny  ki A, Buclin T, Biollaz J et al (2003) NAT2 and CYP1A2 phenotyping with caffeine: head-to-head comparison of AFMU vs. AAMU in the urine metabolite ratios. *Br J Clin Pharmacol* 55:62–67
- Grant DM, Tang BK, Campbell ME et al (1986) Effect of allopurinol on caffeine disposition in man. *Br J Clin Pharmacol* 21:454–458
- Kalow W, Tang BK (1991) Use of caffeine metabolite ratios to explore CYP1A2 and xanthine oxidase activities. *Clin Pharmacol Ther* 50:508–519
- Kalow W, Tang BK (1993) The use of caffeine for enzyme assays: a critical appraisal. *Clin Pharmacol Ther* 53:503–514
- Birkett DJ, Miners JO, Valente L et al (1997) 1-Methylxanthine derived from caffeine as a pharmacodynamic probe of oxypurinol effect. *Br J Clin Pharmacol* 43:197–200
- Fuchs P, Haefeli WE, Ledermann HR et al (1999) Xanthine oxidase inhibition by allopurinol affects the reliability of urinary caffeine metabolic ratios as markers for N-acetyltransferase 2 and CYP1A2 activities. *Eur J Clin Pharmacol* 54:869–876
- Streetman DS, Bleakley JF, Kim JS et al (2000) Combined phenotypic assessment of CYP1A2, CYP2C19, CYP2D6, CYP3A, N-acetyltransferase-2, and xanthine oxidase with the "Cooperstown cocktail". *Clin Pharmacol Ther* 68:375–383
- Chainuvati S, Nafziger AN, Leeder JS et al (2003) Combined phenotypic assessment of cytochrome P450 1A2, 2C9, 2C19, 2D6, and 3A, N-acetyltransferase-2, and xanthine oxidase activities with the "Cooperstown 5+1 cocktail". *Clin Pharmacol Ther* 74:437–447
- Ma JD, Nafziger AN, Villano SA et al (2006) Maribavir pharmacokinetics and the effects of multiple-dose maribavir on cytochrome P450 (CYP) 1A2, CYP 2C9, CYP 2C19, CYP 2D6,

- CYP 3A, N-acetyltransferase-2, and xanthine oxidase activities in healthy adults. *Antimicrob Agents Chemother* 50:1130–1135
25. Vrtic F, Haefeli WE, Drewe J et al (2003) Interaction of ibuprofen and probenecid with drug metabolizing enzyme phenotyping procedures using caffeine as the probe drug. *Br J Clin Pharmacol* 55:191–198
26. Rengelshausen J, Göggelmann C, Burhenne J et al (2007) Reduction in non-glomerular renal clearance of the caffeine metabolite 1-methylxanthine by probenecid. *Int J Clin Pharmacol Ther* 45:431–437
27. Tang BK, Kadar D, Kalow W (1987) An alternative test for acetylator phenotyping with caffeine. *Clin Pharmacol Ther* 42:509–513